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Chemical Constituents of Meat Extracts

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CHEMICAL CONSTITUENTS OF MEAT EXTRACTS

BY

FRANK LEWIS LYMAN

THESIS

FOR THE

DEGREE OF BACHELOR OF SCIENCE IN CHEMISTRY

IN THE COLLEGE OF SCIENCE

UNIVERSITY OF ILLINOIS

1901

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May 31st, 1901

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

F. D. Lyman under Dr. Grindley

ENTITLED Chemical Constituents of Meat Extracts.

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE

OF B. S. in Chemistry.

Arthur W. Palmer

HEAD OF DEPARTMENT OF Chemistry.

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CHEMICAL CONSTITUENTS OF MEAT EXTRACT.

Chemical analysis of meat extracts show that from 3-28 % of their constituents are unknown and at present cannot be determined except by difference. The object of this thesis is to separate and determine the nature and properties of these unknown substances.

Up to the present time, no work has been done on this subject in this laboratory. However, it is closely allied to the subject of meat broths on which a considerable amount of work was done by Mr. H. C. Porter and Mr. Harry McCormack in their theses for degree of M. S. and which at present is being carried on by Dr. Grindley and Mr. Emmett. No record has been found of any investigation upon the subject of meat extracts but quite extensive work has been done abroad by such men as Konig, Bomer, Stutzer and others, whose work will be reviewed below.

Analysis of meat extract show that it contains water, mineral and organic substances. The mineral substances include phosphoric acid, potash and chlorine,while the organic substances consist of nitrogen in the form of unchanged albumin,coagulable albumin and meat; nitrogen as meat bases soluble in alcohol including creatin, creatinin and carnine; also leucine, tyrosine, and other decomposition products, pancreas and albumose peptone and nitrogen as meat bases insoluble in alcohol.

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In the manufacture of meat extracts the name of Liebig is inseparably connected with a certain class of these products. Although he was the first to show how a commercial success could be made of it, he was not the first to propose the concentration of the soluble part of meat. von Petten kofer conducted the first experiments in Munich in 1850--1852, from which finally the manufacture was commenced on a large scale at Fray Bentos in Uruguay.

Liebig's original method consisted of soaking the finely divided flesh in eight times its weight of cold water, filtering the liquid from the insoluble fibre, heating it to coagulate the dissolved albumin, filtering this off and concentrating the filtrate by evaporation to a syrup. However, in practice, it was found necessary to employ a higher temperature for the extraction; the flesh, in a fine state of division, was mixed with the required amount of cold water(free of $CaSO_4$) and the mixture gradually heated to 180°F. As from 30--35 lbs. of lean meat are required to produce 1 lb. of meat extract(freed from albumin and fat) and the amount of lean meat in a cow only amounts to some 300--350 lbs. it was obvious, at first, that meat extract could not be profitably manufactured in Europe. (Chem. News,V. XIV. P. 226. J. Soc. Chem. Jrl. V. XII. P. 370.)

In this country, the preparation of the extract at present is kept a secret, so that only the operation in general is known, which is very similar to Liebig's method mentioned above. The beef is freed of fat and bones(J. B. Chem. Tech. V. XIX. Am. Chem. Jrl. V. II. P. 144.) washed thoroughly and cut

up into proper sizes. It is next introduced into a vat with equal weight of water and one fifth its weight of HCl and cooked fifteen to twenty hours. It is then ground again and cooked 15--20 hours more, then neutralized with Na_2CO_3 and concentrated. Another method is to allow the finely cut beef to stand for a few hours in cold water, then boiling the liquid for a time and afterwards evaporating down in a vacuum pan. In some places, the mince-meat is strained and the resulting liquids evaporated upon rapidly revolving steel plates. In other establishments superheated steam is employed under pressure; the material is then submitted to powerful hydraulic compression and the expressed liquid dried in vacuo.

In reviewing the work done on meat extracts, it might be well to start with the work ofStutzer, for he has probably done more than any other one man upon this subject. He says, (Analyst, V. XX.P.182) that the value of a meat extract as a food material depends on the amount of peptone present, the albumose peptone possessing greater nourishing power than the other. Its value as a stimulant depends especially on the quantity of flesh bases and decomposition products soluble and insoluble in alcohol Gelatin should be regarded as a worthless constituent and should be removed from meat extracts as completely as possible. In the Zeit anal. Chem.V. XXXIV.p.568--570, he remarks that the chief difficulty in the examination of these articles is the determination of the nitrogen in the form of gelatin. He found (Jour. Chem. Soc. V.LXVII. p.543) contrary to former statements that gelatin pepton was insoluble in absolute alcohol. He also found that meat extracts contained ammonia, but was uncertain as to the state of combination.

Stutzer(Analyst, V. X. p.57) first established the nature of the organic and mineral ingredients of meat extracts. He determined, for instaance, what proportion of the nitrogen contained in these preparations must be credited to the easily digested albumen and to peptone; and then estimated in the usual way, by multiplying by 6.25, the quantity of albumen and peptones. He thinks that it would be right to put upon the latter a very special value, because upon them depends pre-eminently the physiological nutritive value of all articles of animal origin. In the next place, he takes into consideration the quantity of nitrogen present in the form of meat bases,(such as creatin, carnine,etc.) because these bases, together with potash and phosphoric acid possess a very high importance in stimulating the nervous system and also for flavoring purposes.

The work of Stutzer has consisted principally in finding out the best methods for the determination of the different constituents in meat extracts. I will give in the following, a brief outline of his methods, which are to be found in the Jour. Chem.Ind. V. XIV. p. 897. They are the result of years of careful experimenting.

In the determination of water, ash, sodium chloride and total nitrogen, he took 5--7 grams of the dry extract and 20--25 of the fluid, weighed into a tinfoil capsule, dissolved in a little hot water, and a weighed amount of fibrous asbestos is added to absorb the liquid, the whole is then dried until constant weight. The ash and total nitrogen are determined in the usual way. The residue from the above is used for the determination of gelatin.

Nitrogen in the form of unchanged albumin, coagulable albumin and meat, was then determined. By using the microscope, the presence of finely divided meat is detected; if found, a suitable quantity of extract is treated with cold water and nitrogen is determined in the insoluble residue. This latter contains only a very small quantity of other albuminoid matter. Acetic acid is added to filtrate from the above and the solution boiled and filtered. The determination of nitrogen in the precipitate gives the coagulable albumin.

In order to determine the nitrogen in the form of ammonia salts--a weighed quantity is dissolved in water and distilled with the addition of $BaCO_3$.

To obtain the gelatin nitrogen, the tinfoil capsule, from the first determination, with its contents, is cut into narrow strips, which are introduced into a beaker and washed four times with absolute alcohol. The residue is then treated twice with ice cold water containing 10 per cent. alcohol, stirred for some minutes, filtered, and the insoluble matter washed with ice cold water until a colorless filtrate is obtained. The residue containing the gelatin is then boiled with water, filtered, and after concentrating the filtrate, a nitrogen determination is made.

He next determined nitrogen in the form of meat bases soluble in alcohol, also leucine, tyrosine and other decomposition products. To an aqueous solution(25c.c.) of the preparation, 250 c.c. of absolute alcohol are added, the solution filtered after 10--12 hours and the residue repeatedly washed with alcohol. The solution contains leucine, tyrosine, and other decomposition

products, together with a portion of the meat bases. The alcohol is distilled off and the residue dissolved in water, the clear solution then made up to 500 c.c. 100c.c. are then taken for the determination of total nitrogen and a second volume of 100 c.c. for nitrogen present as ammonia, the difference being due to the meat bases and decomposition products.

The above residue insoluble in alcohol is treated with water and filtered, the residue containing a small portion of the albumose rendered insoluble by the action of the alcohol which should therefore be extracted with hot water and a nitrogen determination made. The filtrate from the above is made up to 500 c.c. and of this, 50 c.c. are used for total nitrogen, another 50 c.c. for albumose gelatin and peptone and 100 c.c. for determination of peptone. The remainder is concentrated to a small bulk and employed for qualitative detection of true peptone, thus;--An excess of $(NH_4)_2SO_4$ is added and the precipitate of albumose and gelatin filtered off, the filtrate then being treated with a very dilute solution of $CuSO_4$ and an excess of concentrated caustic soda or potash is added, when the characteristic red coloration is produced. The pancreas peptone is obtained by concentrating 100 c.c. of the above solution to 8--10 c.c. and when cold, mixed with at least 100 c.c. of a saturated solution of $(NH_4)_2SO_4$. The precipitate is collected, washed with $(NH_4)_2SO_4$ solution, dissolved in boiling water and evaporated almost to dryness with the addition of $BaCO_3$. The residue is treated with water, filtered and from the amount of nitrogen found in the filtrate, the pancreas peptone can be obtained.

In the determination of albumose peptone, 50 c.c. of

the solution mentioned above is mixed with an equal volume of H_2SO_4 (1:3) and phosphotungstic acid is added until no further precipitate occurs. The precipitate is washed with dilute H_2SO_4 and nitrogen determined. It consists of albumose and pancreas peptone, and gelatin, and as the two latter have already been estimated, the difference will represent the albumose.

The nitrogen in form of meat bases insoluble in alcohol is represented by the difference between the total nitrogen in the solution from the residue insoluble in alcohol and that contained in the phosphotungstic precipitate in the determination of albumose peptone.

In another place (Jour. Soc. Chem. Ind.V.6,p.386 and Zeit. anal. Chem. V. 34, p. 568) Stutzer states that the estimation of gelatin in meat extracts is quite an important one and claims the superiority for his method. It is given here more completely than in the article just reviewed. The extract is dissolved in a sufficiency of hot water in a dish of tinfoil and the solution mixed with enough calcined sand to absorb the liquid. The mixture is then ground, the dish cut in strips and placed in a beaker where it is extracted four times;each time with 100c.c. of absolute alcohol, the supernatant alcohol being passed through an asbestos filter, so as to bring as little as possible of the solids on the filter. After cooling in ice, further extraction is effected by means of a mixture of 100 grams alcohol, 300 grams ice and 600 grams of cold distilled water, at a temperature not exceeding 5°C. The extraction is completed after several times, when the liquid is no longer colored, a separate filter is

employed for each operation and these are afterwards boiled repeatedly with water, the residues being united and concentrated, and the gelatin estimated therein.

In the Analyst V.21, p. 16, Zeit. Anal. Chem. V.34, p. 562--571, and Jour. Chem. Soc. V. 70, p. 83, A. Bomer tells about his experiments on $ZnSO_4$ as a precipitant for albumoses. The precipitation of albumoses by saturated salt solutions and by alcohol depends on the attraction of these reagents for water. So, $(NH_4)_2SO_4$ which is soluble in cold water in the proportion of 76.8 parts per cent. is especially suitable for the purpose. The great disadvantage in its use, is the introduction of ammonia, which must be removed before the nitrogen in the precipitate can be determined. Of the readily soluble $ZnSO_4$ appeared most promising to the author, its solubility being 135 parts in 100 of cold water. So experiments were made to find out whether it could take the place of $(NH_4)_2SO_4$. The precipitations were conducted exactly as in the case of $(NH_4)_2SO_4$method, the precipitate being washed with a cold saturated solution of $ZnSO_4$, 1 c.c. of dilute H_2SO_4 (1:4) was added to prevent the precipitation of $ZnSO_4$. The results obtained corresponded very closely to those obtained by the $(NH_4)_2SO_4$ method, and in no case could the biuret reaction be obtained in the filtrate, showing that the albumoses are completely precipitated by $ZnSO_4$, a further advantage of $ZnSO_4$, is that the peptones, flesh bases, etc. in the filtrate may be at once precipitated with phosphotungstic acid which is not possible in the ammonium sulphate method, as ammonia itself is precipitated by the reagent.

Mr. Bomer has done a considerable amount of his work on extracts with Mr. Konig. The following is a review of some of their work on the composition of meat extracts, the articles were published in the following journals:--Jour. Chem. Soc. V.70 p.82, The Analyst V.21, p.17, Zeit.Anal. Chem. V.34, p.548-562. Jour. Soc. Chem. Ind. V.15, p.130. While there can be no doubt that nearly all the constituents of the muscular fibre which are soluble in cold water will be found in the meat extract, the presence of gelatin or the other decomposition products of the nitrogenous matter is by no means a certainty. Since the extract is prepared at low temperatures, and is only at the end concentrated to the required consistency after filtration, the amount of gelatin can only be excessively small. E. Beckmann's experiments confirm this statement. He could only find .6% of albumin and gelatin in Liebig's extract by precipitation with formalin. On the other hand Kemmerich endeavored to prove that in the South American extract, there was about 6% gelatin and about 30% of albuminoids in form of albumoses, peptones and other soluble compounds. In his analysis, he employed fractional precipitations with alcohol of different strengths, as well as precipitation with $(NH_4)_2SO_4$ and sodium phosphotungstate. The authors critically examined the work of Kemmerich.. The differences were too great, to be accounted for by variation in the extracts, so must have been due to difference in method, Kemmerich having determined the amount of his precipitates graviametrically and not by direct estimation of nitrogen.

Regarding the chemical examination of meat extracts of which they have made an extended study, the authors

remark:--

1. Precipitation with 80% alcohol is of no value in determining the kind of nitrogen.

2. Albumoses should be determined by salting out with $(NH_4)_2SO_4$ or $ZnSO_4$.

3. The filtrate from the $(NH_4)_2SO_4$ or $ZnSO_4$ precipitates should be decolorized with animal charcoal and tested for peptones by the biuret reaction.

4. A determination of the ammonia by distilling an aqueous solution of the extract with ignited magnesia is valuable.

5. When peptone has been proved to be absent, the nitrogen in the phosphotungstate precipitate, after deducting the nitrogen derived from gelatin, albumoses and ammonia may be ascribed to the flesh bases. The precipitate should stand at least one day.

6. The difference between the total nitrogen and the nitrogen in the form of gelatin + albumoses + flesh bases + ammonia gives the amount of nitrogen present in compounds not precipitated by phosphotungstic acid.

In his researches on the composition of meat extracts (Chem. News. V.76, p.35) J. Bruylants says,--It has long been admitted that extracts of meat contain, as proteic substances, but a small quantity of gelatin. A large proportion of the nitrogen belongs to other proteic substances, such as albumoses and peptones. That is to say, substances whose nutritive value is greater than that of albuminoides, since they have already undergone some of the modifications due to digestion.

Mr. Weidel and Mr. Voit seem to both have discovered carnine, a new base in meat extract. While carrying on a series

remark:--

of experiments on extracts of meat, H. Weidel discovered a new organic base, Carnine, (Chem. News. V.24, p.35.) present to about 1 % in the preparation. In the pure state, it is a crystalline solid, insoluble in cold, readily soluble in boiling water and insoluble in alcohol and ether. Carnine has a bitter taste, a neutral reaction and is not precipitated by neutral lead acetate. The formula is $C_7H_8N_4O_3$ ~~having~~ being different from the formula of the bromine, $C_7H_8N_4O_2$, by only one atom of oxygen. It has a large series of salts.

In the Jour. Chem. Soc. V. 24, p.715, Mr. Weidel has another article on carnine. The supposed nutritive powers of meat extract lead naturally to the supposition that creatin and creatinin are the substances that give it these powers. However, Mr. Voit, having maintained that this action of meat extract does not arise from the presence of these bodies, experimented on a large number of extracts, which resulted in the discovery of the new base, carnine. The extract was dissolved in to six or seven parts of warm water and carefully precipitated with strong baryta solution, avoiding excess, the mass being filtered through a linen cloth and the filtrate precipitated by basic lead acetate. A lead compound of carnine is thus thrown down and being soluble in boiling water can thus be separated from the other bodies likewise precipitated. From this solution, it is then separated and obtained pure. Doses of 1/2 to 2 decigrams of carnine and its hydrochloride appear to have a slight effect on the nervous system, a slackening of pulsation being the most marked symptom.

There is a prevalent, but very erroneous opinion, says Dr. Thudichum, (Chem. News, V. 15, p.161) that extracts of meat are nutritious and can be used as a substitute for meat. Manufactures claim on behalf of these preparations that the various additions and methods of treatment give them value as real foods. This is true in but a very limited sense, for the amount of such preparations which would require to be taken to furnish the carbon and nitrogen requisite to support life is enormously beyond the quantity of any of the preparations which could be consumed with out upsetting the system. Besides this, there is the extravagant cost of meat extract, if used in quantities necessary to sustain life.

In judging of the amount of credence to be attached to statements regarding the nutritive value of extracts, it should be borne in mind that fresh lean meat contains about 20 per cent of nutritive value and 75% water. By the dessication of four lbs. weight, there will be obtained 1 lb. of dry substance of which 80 per cent is nutritive proteid matter, the remaining 20 per cent

consisting of fat, meat bases, salts etc. By no possible means can further material concentration of the nutritive matter be effected. So that statements, that meat extracts contain the nutritive matter of thirty, forty or fifty times their weight of fresh meat, are not justifiable.

According to Liebig's own statement meat extract, like tea and coffee serves as a strong stimulant of the nerves of the heart and of the brain, increases the secretion of saliva and aids digestive processes. Because of its similar effects, it

must contain a substance which is somewhat similar to those contained inthe above mentioned stimulants. Too strong a solution of extract of meat is just as injurious as too strong tea or coffee. The important effect of these preparations, i.e. their stimulating powers, seems to be due to creatin and allied substances, the action of which in some degree resembles that of theobromine.

In the Am. Chem. Jnl. V. 20, p. 869, Mr. Ladd and Mr. Bottenfield give three methods for the separation of creatin. They first explain the method of Nebauer's in which the meat is first cut into small pieces by running through a sausage machine. 700 grams of this product is then extracted for 10-15 minutes with an equal weight of water at 55-60° C. It is then pressed and again extracted with water as before. The two extracts united are then heated to boiling in order to coagulate the albumin. After filtering, the filtrate,is treated with basic lead acetate as long as a precipitate forms, the excess of lead being removed from the filtrate by hydrogen sulphide. The filtrate is now carefully concentrated to a small volume and allowed to stand for several days, so that the creatin may crystallize out. These crystals are collected on filter paper and well washed with alcohol. They are redissolved, filtered through charcoal and again recrystallized, well washed with alcohol, dried and weighed.

In Liebig's method, the product was ground in a sausage machine and then extracted with 1/2 half its weight of cold water for a few hours. It is then pressed with a screw press for several hours and again extracted in the same manner with cold water.

The two filtrates are united and heated to boiling to coagulate the albumin. Baryta water is added to the filtrate to remove the phosphates, the excess of barium being removed with CO_2. After filtering and washing, the filtrate is evaporated on a water bath to a syrupy consistency and allowed to stand several days. The crystals of creatin settle out and are purified as before.

Stradeler's method consists of digesting the finely divided material with twice its volume of alcohol, pressing as before and evaporating the filtrate. A solution of basic lead acetate is then added as long as a precipitate is formed and the process is continued as before.

Results show that Nebauer's method give figures somewhat higher than Stradeler and Liebig. There are several sources of error in Liebig's method. The use of cold water does not leave the meat in as good condition as in Nebauer's method and it is impossible to press the meat as dry. Again they were not able to remove all traces of the barium by means of CO_2 without some loss of creatin The use of alcohol in Stradeler's method results in extracting some of the fats and other products, and a removal of these products results in some loss of creatin; but this is not as great as in Liebig's method.

Creatin has the formula, NH. $C(NH_2)$, $N(CH_3)$ CH_2. COOH + H_2O and is methyl guanadin acetic acid. It crystallizes in hard colorless, shining monoclinic prisms, containing one molecule of water of crystallization which it loses when heated to 100° C.

It dissolves in 74 parts water at the ordinary temperature and in 7400 parts parts absolute alcohol. It is insoluble in ether. Its watery solution is neutral in reaction and has a bitter taste. Creatin forms crystalline compounds with mineral acids and mercury. When heated with dilute mineral acids, it is converted into creatinin. It forms compounds with certain metallic solutions.

Besides the stimulating powers of creatin etc., there are the potassium salts, which the body requires for the production of muscular power, potash being as essential an element in the Chemistry of muscles as in that of the blood. Then there are certain acids such as phosphoric and inosic which produce a relishing flavor, that of meat.

Although albumin has a definite food value, the quantity here is too small to be of service -- at the same time it reduees the value as a preparation as a stimulant -- and so is removed from the extract. Certain extracts have an addition of

albumin, but it can scarcely be regarded as of any material service. Some producers add flesh powder--with or without albumin--with the object of giving them some definite food value. As this does not amount at most to more than 8-10 per cent, it is obvious that a large quantity of the substance would be required to obtain as much unaltered proteid as is contained in an egg. On the other hand, it has been demonstrated, that there is nothing to show that flesh powder suspended in meat extract is more digestible than ordinary flesh in the same fine state of division. Besides the amount of flesh bases, the principal stimulating agents is correspondingly reduced.

When meat extract is manufactured on a commercial scale, care is taken to extract the flesh at as low a temperature as practicable in order to prevent the formation of gelatin from the coleagene. Although gelatin is not valueless, its food value is of a very different character to that of albumin. Its function in the system is to save the abluminous substances which would otherwise be oxidized with the formation of heat, but it is quite unable to replace the nitrogen daily lost by the disintegration of the cells of the body. For example, Voit, (Zeit. Biol. 1884, p.284) experimented on a hungry dog, which lost 5.3 grams of nitrogen per day but by giving it gelatin, the daily loss sank to 2.1 grams. The latter quantity represented the loss from the organic nitrogen of the cell decomposition, and it was found that by adding that amount of albuminous nitrogen to the gelatin, equilibrium was established.

When analysing these preparations, it is usual to calculate all of the chloride to NaCl, though the greatest proportion of the naturally occuring chlorides in flesh consists of

Three commercial cheese processors, it is among the outstate all of the eligible to [illegible] the standard pasteurization of the material) remaining eligible to [illegible] accounting for 3.1 billion. The latter quantity represents the 3000 plus the regional milkshed of the only Wisconsin's [illegible] 8.3 cheese of milkfarms [illegible] by 1970 it [illegible] (Hall. Ohio, 1964, p.314) [illegible] as a handful down, during fluorescence of the milk by the [illegible]. Dr. Hamlin, Hall. [illegible] milk system is [illegible] the dairy [illegible] milkshed [illegible] milk [illegible] [illegible] [illegible]

KCl. In order to calculate the amount of added salt, Allen(Commercial Organic Analysis, V. 4, p. 303) makes an allowance of .06% of chlorides as NaCl for each unit per cent of solid matter present and subtracts this from the total chlorine as NaCl formed in the extract. Some manufactures add sodium chloride, but Liebig stated that this was not required, as it is manifest that like added meat fibre, such an addition must lessen the proportion of meat bases.

It appears, therefore, that meat extracts have a true value as stimulants and restoratives, the proportion of meat bases, extractives and salts present being an index of their value in this respect. On the other hand, all attempts to give them the characters of true nutritive concentrated foods can meet with but a very limited success. A failure to appreciate these facts has caused very delusive values to be placed on such preparations. The errors have been further increased by the discordant methods of judging of the value of such articles.

Thus, Stutzer expressed the opinion, as stated before that albumoses and peptones are the only constituents of value in a meat extract, ignoring any meat fibre, gelatin or coagulated albumin which may be present. Another analyst regards the matters precipitated by alcohol as being the only constituents of value. Such a contention, however, is clearly insupportable, as the precipitate formed by alcohol, contains a variable but very considerable per cent of non-nitrogenous extractives and salts. On treating an aqueous solution of Liebig's extract of meat with excess of strong alcohol, Allen obtained a precipitate weighing 31.8 per cent of the original extract and containing 11.7 per cent

ash. The nitrogen in the precipitate corresponded to 10.6 per cent of proteids leaving 9.5 per cent for non-nitrogenous matters.

A recent analysis of meat extracts by Hehner(Allen's Organic Analysis, V. 4, p. 311) shows the composition of a number of well-known preparations. They were made by a method essentially the same as that of Stutzer. Each of the nitrogenous constituents was calculated from the nitrogen, as determined by Kjeldahl's method, the factor 6.25 being used in every instance.

Description	Water	Fat	Gelatin	Meat fibre and Coag. Alb.	Albumoses	Peptones	Meat losses	Ash	Difference	Sodium-Chloride	Phosphoric acid.	Total N.
Liebig Co's Ext. carnis.	15.26	0.34	5.18	2.12	2.01	8.06	29.32	23.51	4.20	5.81	6.97	9.07
Armor's Ext.	15.97	0.21	3.31		1.75	5.13	41.12	29.36	3.15	9.74	6.76	8.21
Brand & Co. Ext. carnis.	17.85	0.38	4.56	1.81	4.19	10.16	38.90	18.80	2.87	3.31	5.16	9.80
Liebig's Ext. (Bovril's & Co. make)	22.24	0.29	5.50	1.30	3.62	8.44	38.58	20.45	.42	5.14	5.50	9.19

The per cents which are the most important to us are those tabulated under difference. They show the amount of

nitrogenous or non-nitrogenous matter contained in meat extracts which is obtained by difference.

In my work on meat extracts in the labor atory, I first obtained a considerable quantity of two well known brands;-viz.- Liebig's and Cudahy's. My object was, to first get rid of the known constituents, chiefly the mineral, and nitrogenous organic matter. Then to make systematic tests upon the substance or substances remaining and to devise a method for separating and purifying the same. These substances when purified were to be identified by analysis and a study made of their chemical behavior and reactions. Then, methods for the quantitative determination of the bodies were to be perfected and the quantities of the materials determined in a number of meat extracts.

I weighed off about 50 grams of the solid extract into a beaker, and treated it with some 200-300 c.c. of water free from nitrogen, stirred thoroughly and allowed to stand several hours with frequent stirring. It was then filtered and the small amount of residue upon the filter paper thoroughly washed with water until the volume of the filtrate approached 500 c.c. The solution was then made up to exactly 500 c.c. This was then analysed for total nitrogen, total solids, ash and albuminoid nitrogen.

The total nitrogen was determined by Kjeldahl's method. Ten c.c. was taken and carefully transfered to an 800 c.c. Schott and Gerossen, Kjeldahl digesting and distilling flask and 0.65 grams of metallic mercury with 25 c.c. of pure conc. H_2SO_4 were added. The digestion was commenced and watched closely at first so as to prevent foaming; after that the acid was brought

to boiling. It generally required about four hours to complete the digestion, after which the oxidation is completed, while yet hot, by adding carefully, powdered $KMnO_4$. While the flasks are cooling, the required amount of acid is measured with an excess of 3-5 c.c., into the receiving flasks. Placed the receivers to the condensers and made sure that the delivery tubes extended entirely to the bottom of the receiving flask. When the digestion flasks are sufficiently cool, I added 200 c.c. of nitrogen free water, 25 c.c. of K_2S solution(40 grams per litre) and shook thoroughly. Three or four pieces of granulated zinc are now added and 80-85 c.c. of NaOH solution(600 grams per litre) running the latter down the sides of the flask so as not to mix with the acid contents. Washed neck of flasks free of alkali, then connected immediately with proper condenser, mixing contents thoroughly by shaking, and distilling as rapidly as possible. When some 175-200 c.c. had been collected in the receivers, ceased the distilling and titrated excess of acid with standard ammonia. The amount of acid used divided by weight of sample times the factors of the acid equals per cent nitrogen and this multiplied by 6.25 equals the per cent of protein.

The total solids were determined by burning the residue from the total solids.

For the determination of albuminoid nitrogen, I took 25 c.c. of the solution and introduced it into a Kjeldahl flask, added 200 c.c. of water free from nitrogen, 10 c.c. of dilute HCl and then bromine little by little with constant vigorous stirring. Saturated with bromine and finally added slight excess, leaving a residue of 2-3 c.c., and allowed to stand over night.

Filtered and washed residue thoroughly with bromine water. The bromine precipitates the proteids while the flesh bases and other forms of nitrogen are left in solution. The amount of albuminoid nitrogen obtained was very slight and as there was an error in the work, the result will not be given below.

The following are the results:-

Description.	% Total Solids	% Water	% Ash	% Total Nitr.	% Creatin	% Other Sub.
Liebig's Ext. of Meat.	71.01	28.99	26.14	7.19	22.43	22.44
"	71.08	28.92	26.27	7.21	22.49	22.32
Cudahy's Ext. of Meat.	72.98	27.02	26.52	5.16	16.10	30.36
"	73.12	26.88	26.63	5.04	15.72	30.77

The water was obtained by difference while the per cent of creatin was determined by multiplying the total nitrogen by 3.12. On subtracting this amount together with the ash, from the total solids, the per cent of other substances, by difference was obtained.

These analyses indicate that there is a large amount of substances undetermined in these extracts, by the ordinary method used in the analysis of foods. The results also show, Cudahy's Extract of meat has a larger amount of unknown substances in it than Armour's Extract. This being the case the following analyses was undertaken.

The contents of one can of Cudahy's extract of beef was weighed and transfered to a 1-1/2 -2 liter flask. An extraction was made with boiling 95 per cent alcohol, repeatedly using about 750 c.c. each time. After each extraction, the contents of the

Description.	Total Solids	Water	Ash	Total Fibre.	Crude Protein	Sugar
Liebig's Ext. of Meat.	73.03	26.97	24.14	7.12	23.15	
	73.04	26.96	24.21	7.13	23.47	
Cambay's Ext. of Meat.	73.80	27.03	24.08	8.16	17.31	
	73.12	26.88	24.03	7.04	16.19	

flask were allowed to stand a few minutes and then the liquid decanted carefully into a two liter flask. As soon as two liters of the extract was obtained, the alcohol was distilled off and used for extracting the insoluble residue again. Poured the extract thus obtained into the same two liter flask and continued the extraction until 750 c.c. of the extract contains not more than one gram of solids.

Having completed the extraction in this manner, diluted the extract obtained by evaporation of the alcohol, to a definite volume--1000 c.c. Mixed thoroughly and measured with a burette exactly 25 c.c. of this solution and diluted with water, free from ammonia, to 250 c.c. Mixed thoroughly and determined in this dilute solution:--Total solids and ash, taking 25 c.c.; total nitrogen, taking 10 c.c. and albuminoid nitrogen taking 25 c.c. The determinations were conducted as explained before, with the exception in the case of albuminoid nitrogen, in which 5 c.c. of dilute HCl was used in place of 10 c.c.

I then took 100 c.c. of the original solution and added $Ba(OH)_2$ solution (50 grams in 1000 c.c.) until slightly alkaline to litmus paper. It was then allowed to stand over night and as a precipitate was formed, this was filtered off and washed with a little water. The filtrate was then made up to 250 c.c. and analyzed, determining total solids, total nitrogen and ash as before.

One hundred c.c. of this last filtrate was then taken and basic lead acetate added in slight excess. The basic lead acetate was made by dissolving 170 grams of lead acetate in hot water, adding 100 grams of lead oxide (PbO) boiling for half an hour, diluting to 1000 c.c. and filtering. After the addition

of basic lead-acetate, the solution was allowed to stand over night, when a precipitate formed which was filtered off and washed. The excess of Pb was removed wtih H_2S which was filtered and washed. The filtrate was then made up to 250 c.c. and determinations of total solids, total nitrogen and ash made .

A sample of Armour's meat extract was analyzed in the same way.

The object of adding the $Ba(OH)_2$ and basic lead acetate is to get rid of as much mineral and nitrogenous organic matter as possible, so as to leave in solution if possible the unknown substances sought. The $Ba(OH)_2$ takes out the phosphates together with the sulphates and perhaps lactic acid. The basic lead acetate removes the creatinin and remaining proteid compounds.

The results of this work are presented in the following table.

Cudahy's Ext.	% Total Solids	% Ash	% Total Nitr.	% Albuminoid Nitrogen	% Creatin	% Other Sub.
Water Ext.	39.45	7.49	3.104	.0771	9.68	22.28
Water Ext.	39.49	7.79	3.067	.1147	9.57	22.13
After Addition of $Ba(OH)_2$	43.14	8.53	2.571		8.02	26.59
" "	43.10	8.33	2.547		7.94	26.83
After Addition of Pb acetate.	29.48	9.41	2.475		7.72	12.35
" "	29.30	9.20	2.468		7.70	12.40
Armour's Ext.						
Water Ext.	43.86	6.25	6.109	.00436	19.06	18.55
Water Ext.	44.00	6.04	6.109	.00436	19.06	18.90
After Addition of $Ba(OH)_2$	46.42	9.73	4.065		12.68	23.91
" "	46.24	9.94	4.320		13.47	22.83
After Addition of Pb acetate.	30.77	9.95	3.893		12.15	8.67
" "	30.55	10.13	3.995		12.46	7.96

From the above table, we see that, after the addition of $Ba(OH)_2$ the total solids and ash increased, instead of decreasing as we would expect. This was probably due to the fact I neglected to remove the excess of barium with CO_2. The total nitrogens in both cases have decreased. After the addition of lead subacetate, the analyses show that the total solids, ash and total nitrogen, in the case of both the Cudahy's and Armour's extract, have all decreased which goes to prove that they do remove the mineral and nitrogenous organic bodies to a certain extent. However, they were not taken out as completely as was expected, so another

Company's Name.	£ Initial Outlay	£ Loss	£ Total Capital.	£ Authorised Capital.	£ Remarks	£ Other Pay.
Water Bds.	89.15	7.89	3.344	.2771	9.69	23.19
Water Bds.	39.69	7.79	3.087	.1147	9.87	28.13
After Addition of Pay(2nd)	42.14	8.59	3.971		8.18	31.69
" "	41.19	8.74	3.317		7.84	33.34
After Addition of 3% Capital.	39.88	1.13	3.479		7.71	32.17
" "	38.39	8.19	3.411		7.71	32.69
Ashmore's Bds.						
Water Bds.	49.81	8.89	1.319	.00838	14.09	34.74
Water Bds.	41.79	8.14	3.319	.00438	33.84	34.77
After Addition of Pay(2nd)	41.89	7.73	8.911		33.69	33.31
" "	34.19	8.84	3.881		33.87	29.83
After Addition of 3% Capital.	33.77	8.98	3.913		33.38	8.87
" "	31.84	31.13	3.891		32.84	7.11

The results of this work are presented in the following table.

Among the above tables, we see that, after the addition of Pay(2nd) the total outlay was increased, instead of decreasing as we would expect. This was probably due to the fact that it was necessary to remove the service of Darling with CO$_2$. The total diminution in hydrochloric here observed. After the addition of 3rd capital, its results show that the total outlay, but some total outlay, in the case of both the Company's and Ashmore's waters, have all decreased which goes to prove that they do remove the ammonia and nitrogenous products bodies to a certain extent. However, they were not taken out as completely as was expected, so another

method was employed.

I next took four, two ounce cans of Armour's extract of beef and weighed the contents carefully into a beaker, adding 500 c.c. of distilled water free from nitrogen, stirring thoroughly and warming to about 80°C. It was next cooled down and a saturated solution of AgCl added as long as a precipitate formed. This was allowed to stand over night when it was filtered on a Buchner funnel and the precipitate washed with a little water. The excess of mercury in the filtrate was removed with H_2S, the precipitate of HgS being washed with a little water.

To this filtrate, I added $PbCO_3$ until the solution was nearly or quite neutral. This was again filtered on the Büchner funnel and washed with a little water. The filtrate was treated with H_2S to remove the excess of lead. This filtrate was then made up to 1000 c.c. and the total solids, ash and total nitrogen determined.

Trouble was found here in obtaining the total solids and ash. On testing with litmus, it was found to be decidedly acid. So a further precipitation with freshly precipitated Ag_2O was made and filtered and the excess of Ag removed with H_2S. The freshly precipitated Ag_2O was made by adding KOH in excess to a saturated sol'n of $AgNO_3$ filtering and washing the Ag_2O with dilute KOH, then with boiling water until all nitrogen was removed. The filtrate from the Ag_2O precipitation was made up to a definite volume and the total solids, total nitrogen and ash determined.

The addition of $HgCl_2$ takes out principally, the creatinin. When this filtrate is concentrated, it is strongly acidified with HCl which has been formed on the addition of H_2S.

This would tend to change the creatin into creatinin, which would probably be precipitated on addition of the $PbCl_2$. However, the primary reason for introducing the $PbCO_3$ and finally the Ag_2O is to get rid of the excess of HCl which would be thrown down as $PbCl_2$ and AgCl.

The results were all determined as before with the exception of the ash. Because of the substances present in the total solids which were volatile at a red heat (such as KCl), it was first charred, and the soluble part extracted with nitrogen free water. The residue was then burned white, the solution of the soluble part added, evaporated, heated in a water oven and then ignited at a low temperature for a few minutes. This is the "Official Method" for the determination of ash.

The results are presented in the following table.

Armour's Ext.	% Total Solids	% Ash	% Total Nitrogen	% Creatin	% Other Sub.
After Addition of $HgCl_2$ and $PbCO_3$			2.906	9.067	
" "			2.901	9.051	
After Addition of Ag_2O	52.33	21.37	2.906	9.067	21.89
" "	51.53	21.60	2.896	9.035	20.89

In comparing this with the foregoing table, it shows that the total nitrogen has decreased, but that the total solids and ash have increased. On account of lack of time the work was dropped at this point. The results show plainly that there is a considerable quantity of substances in meat extracts which is not determined by the ordinary methods of analysis. However the separ-

Lime Addition of $MgCl_2$ and $PbCO_3$			8.904	0.187
" "			8.893	0.083
Lime Addition of $MgCl_2$	84.32	41.37	8.894	0.087
" "	83.58	41.80	8.898	0.088

ation, identification and study of this substance or these substances have not been accomplished by the writer.

"Approved"
H. S. Grindley,
Associate Professor of Chemistry.

May 30th 1901.

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